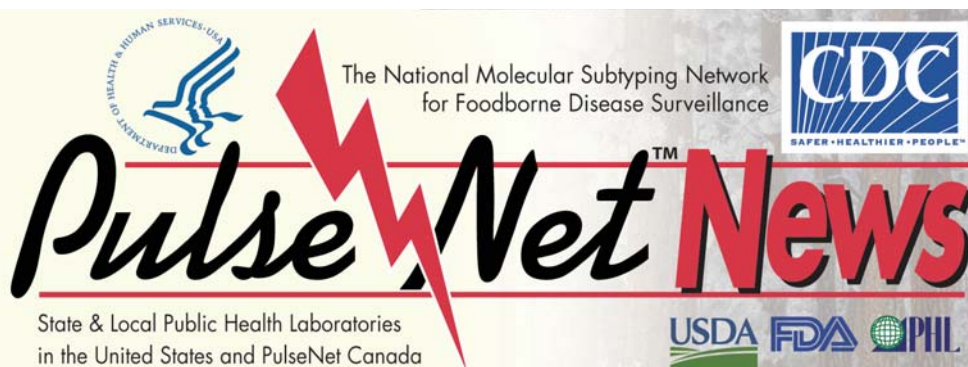


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State & Local Public Health Laboratories
in the United States and PulseNet Canada



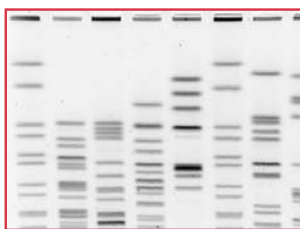
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Certification and Proficiency Testing: Where We Stand Now

Christine Steward, APHL Contractor, PulseNet QA/QC Program

The PulseNet Quality Assurance/Quality Control (QA/QC) Program, which includes certification and proficiency testing of the PulseNet participating laboratories, is growing. The purpose of the program is to allow accurate comparisons of TIFF images within the PulseNet national databases by ensuring that TIFFs of all gel images are comparable, of excellent quality, and have correct normalization and consistent band assignment.

Certification. Currently, certification is a one-time event for each person for each PulseNet organism. Individuals can be certified for "TIFF image only" (i.e., laboratory methods for Pulsed-field gel electrophoresis (PFGE) and image acquisition), "analysis only" (i.e., BioNumerics analysis of gels using PulseNet client scripts and access to the national database), or "TIFFs and analysis". To become certified, laboratorians should send an email to PFGE@cdc.gov requesting the strain set that they wish to have for certification. The number of strains in each set was reduced in 2003 so that only one 10-well gel is required; *Listeria* sets were first distributed in March 2003 and *E. coli* and *Salmonella* in May 2003. A *Shigella* certification set is also available. Each certification set is run on one gel. Once certification gels are produced and/or analyzed by an individual, the TIFF



and/or bundle certification files are emailed from the participating laboratory to PFGE@cdc.gov. We then evaluate the submissions and report back to the submitter. If the submitter is deemed analysis-certified, the individual will receive a SecurID key fob and login for on-line access to that database. The SecurID key fob is assigned to an individual, rather than a laboratory, and thus must only be used by that assigned (certified) individual.

Currently, CDC can provide a maximum of two SecurID key fobs to each certified PulseNet participating public health laboratory, even though there may be more than two certified people in a laboratory.

As of 2003, more than 150 individual certifications were completed, an impressive number of U.S. PulseNet-participating laboratories with individuals who are certified in analysis for *E. coli* O157:H7 (41 laboratories), *Salmonella* (37 laboratories), and *Listeria* (14 laboratories). However, these numbers do not include all U.S. participating laboratories. Compliance with certification procedures is a requirement for all PulseNet participating laboratories; therefore, all laboratorians working in

PulseNet-related activities should be certified. Laboratories with analysis-certified individuals can query and upload their own data to the national databases.

In the near future, a group of individuals from PulseNet-participating laboratories will help create a document detailing a change

(Continued on page 2)

Another Aspect of Real-Time Subtyping

Stephen Dietrich, Laboratory Scientist, Michigan Department of Community Health, Lansing, MI

The importance of real-time subtyping has been emphasized a number of times in PulseNet meetings and in the PulseNet News. An issue associated with real-time subtyping that is not often discussed, but is also important, is the need to test isolates with a second enzyme. A cluster of isolates that is indistinguishable based on a primary enzyme identification can be further discriminated when restricted with the second enzyme. These second enzyme results are often a vital part of epidemiologic investigations.


At the Michigan Department of Community Health, we routinely test every shiga toxin-positive *E. coli*, *S. ser.* Newport, and *L. monocytogenes* with two enzymes. Since the laboratory typically does not receive many isolates of either organism at any given time, we do not feel this is a burden. Clusters of *E. coli* O157:H7 and *S. Newport* are frequently identified, and we find testing with both enzymes on the initial gels is more efficient than running the second enzyme later. Although the degree of clustering of *S. Newport* isolates in Michigan has

(Continued on page 2)

PulseNet In the News

Dr. Frank Plummer and the Winnipeg National Microbiology Lab were recently spotlighted in the *Winnipeg Sun*. The article, "City lab to become centre of new disease tracking system" can be found in the October 15, 2003 issue.

- 1) Satisfactorily complete annual proficiency tests.
- 2) Establish an in-house QA/QC program to verify that individuals are following the standardized protocol and are producing quality work.
- 3) Undergo an annual review of their QA program by their area laboratory.

If your PulseNet-participating laboratory does not have certified individuals, or if you have questions or comments about certification or proficiency testing, contact PFGE@cdc.gov. In any email correspondence pertaining to the above, please include the words "Certification" or "Proficiency Testing" in the subject line. Our goal is for all participating laboratories to be on-line. This will make comparisons with the national database more efficient for all laboratories and will further speed the identification of outbreaks. 



If you have ideas or suggestions for the agenda or would like to volunteer as moderator for any of the sessions, please contact Shari Rolando, APHL at Srolando@aphl.org or (202) 822-5227, ext. 205.



Please begin to consider candidates to be nominated for the PulseStar Awards that are presented annually by FDDL/CDC and APHL. The award consists of a plaque and a check for \$500 provided by APHL. Nomination forms and criteria will be posted on the WebBoard.

# PRIMARY ENZYME CLUSTERS*	# PRIMARY ENZYME CLUSTERS*	DISCRIMINATED WITH SECOND ENZYME
<i>E. coli</i> O157:H7 and O157:NM	56	24
<i>L. monocytogenes</i>	19	10
<i>S. Newport</i>	17	10

Second enzyme testing is probably more important for primary enzyme patterns that are found more commonly. For instance, we have found one *Xba*I pattern in 31 isolates of *E. coli* O157:H7 identified since 1999; 11 *Bln*I patterns were found in 24 (of 31) isolates tested with this enzyme. The above table describes the compiled data on the discriminatory ability of the second enzyme for Michigan's isolates of *E. coli* O157:H7 and O157:NM, *L. monocytogenes*, and *S. Newport*. For each organism, the second enzyme proved to be very good at subtyping primary enzyme clusters. Discrimination occurred for temporally-similar and dissimilar clusters. **CDC**

PulseNet Training Workshop for Food Industry Scientists

Catherine Nnoka, International Life Sciences Institute N.A., Washington, DC

ILSI®
International Life Sciences Institute

ILSI NORTH AMERICA

The Centers for Disease Control and Prevention (CDC), in partnership with the Division of Consolidated Laboratory Services (DCLS), Commonwealth of Virginia, and the International Life Sciences Institute North America (ILSI N.A.) Technical Committee on Food Microbiology recently conducted a PFGE and BioNumerics special four-day workshop to familiarize food industry scientists with PulseNet, CDC's molecular subtyping network for foodborne bacterial disease surveillance.

Twelve scientists, an equal number of instructors and staff from the Association of Public Health Laboratories, CDC, DCLS facility, Food and Drug Administration, and ILSI office in Washington, DC, braved the aftermath of Hurricane Isabel to gather at Virginia's state public health laboratory in Richmond, Virginia, from September 23-26, 2003, to participate in an intensive four-day program that represented a first-of-its-kind collaboration between the government, the public health sector, and the food industry.

Through a program of pulsed-field gel electrophoresis (PFGE) lectures and hands-on laboratory exercises, workshop participants received training in standardized molecular subtyping of foodborne pathogenic bacteria, with *Listeria monocytogenes* as the test organism. Instructors from CDC, DCLS, and Bio-Rad Laboratories addressed all aspects of standardized molecular subtyping, including detailed laboratory protocols, computer acquisition of the PFGE patterns, their normalization and analysis, comparison of the patterns to a database, and interpretation of subtyping data.

One of the scientists who participated in the workshop will return to Cornell University to conduct PFGE subtyping of the isolates in the ILSI N.A. *Listeria monocytogenes* Reference Strain Collection, which is

(Continued on page 5)

PulseNet Laboratory Profile

New Mexico

PFGE laboratory scientists Sonya Flores & Paul Torres



Sonya P. Flores, Laboratory Scientist III, and Pascale M. Léonard, Supervisor, Molecular Biology, New Mexico Scientific Laboratory, Albuquerque, NM

working up the samples will communicate this information to the PFGE technician.

The New Mexico State Lab is located in Albuquerque on the University of New Mexico campus. Officially known as the Scientific Laboratory Division (SLD) of the New Mexico Department of Health (NM DOH), it houses three Bureaus (Toxicology, Chemistry and Biology) as well as the New Mexico Department of Agriculture Veterinary Diagnostic Services - VDS, and The University of New Mexico, Office of the Medical Investigator - OMI. The Biology Bureau includes six sections: General Microbiology, Environmental Microbiology, TB/Mycology, Virology/Serology, Metabolic Screening, and Molecular Biology. These Sections handle various types of samples including clinical hospital specimens that are sent for confirmatory identification and serotyping; public health clinical samples; VDS and OMI samples; environmental samples such as milk, food and water; and bloodspot newborn metabolic screening samples. The laboratory serves as a support service to the Department of Health, Department of Agriculture and the Environment Department.

The SLD uses Microsoft Access as a LIMS system to track samples from all sources and sections. As samples enter the building through the specimen receiving area, they are sorted as the demographic and specimen information is entered into the database. The sample is then transferred to the appropriate section and processed. At the Scientific Laboratory Division (SLD), two laboratory scientists are trained in PFGE. Both scientists process samples, make plugs, and run gels. Only one scientist, the PFGE technician, is in charge of analyzing gels and reporting data which ensures consistency of data analysis.

An enteric sample submitted to the General Microbiology Section is typically identified to serotype. Several scenarios may occur that will result in the identification of an organism implicated in a foodborne outbreak:

1) In the event of multiple samples being identified with the same serotype, the laboratory scientist

2) Sometimes, the Office of Epidemiology is informed by doctors or hospitals of a cluster of patients who exhibit similar symptoms. The Office of Epidemiology will then contact the PFGE technician and the General Microbiology section supervisor to inform them of the potential for epidemiologically connected samples. This sharing of information facilitates the work-up performed by the laboratory scientists and the PFGE technician.

3) On most occasions, an outbreak is detected only after several weeks have elapsed because samples come in sporadically and laboratory scientists do not notice a significant increase in a particular serotype.

4) Lastly, if a rare serotype is isolated a number of times within a short period of time, the PFGE technician will be asked to run those isolates prior to routine PFGE work.

The resulting PFGE patterns are examined rigorously to identify any trends of epidemiological significance. For each sample, the pattern designations are input along with any other results into the Access database. This allows the user to search the database for previous isolates with the same pattern designation, serotype, submitter, dates of collection and receipt. Although BioNumerics is used to analyze gels and produce dendrograms, the Access database is used to query for other samples that had the same pattern designation. The data from such a query provides all the information from each sample, as opposed to the information found in the BioNumerics window where only some of the data fields are present in an observable view. Also, the Access database is used to create reports that are sent to both CDC and the Office of Epidemiology.

If a serotype cluster results in identical pattern designations, the Office of Epidemiology is immediately informed. The Office of Epidemiology was recently alerted when a rare *Salmonella* serotype was identified (Kiambu) from beef jerky packaged in New

Mexico. Early in the suspected outbreak, all new samples of the same serotype coming into the lab were run with priority on PFGE. In addition, we notified CDC PulseNet via the PFGE mailbox and posted on the WebBoard to see if this pattern was identified in any other states. The Office of Epidemiology interviewed every patient from whom samples had been obtained, but at the beginning of the investigation were unable to pinpoint a single source of infection. Back at the Laboratory, a second restriction enzyme was used in the PFGE analysis to see if the presumed single source of infection was possibly multiple sources. We obtained no greater discrimination of the patterns from the new analysis. The Office of Epidemiology conducted more interviews with patients, and finally determined a single food source associated with the infection. The food source was beef jerky.

The Office of Epidemiology continued to work with other state and federal agencies including the Albuquerque Environment Department, USDA/FSIS, and FDA. Beef jerky samples arrived at the laboratory and were processed by the Environmental Microbiology Section to isolate any potential foodborne pathogens. Stool samples from people who worked at the beef jerky processing plant were sent to the General Microbiology Section. If *Salmonella* Kiambu was isolated, PFGE was run immediately to corroborate the epidemiological investigation. The outbreak was identified within a short period of time, and the Office of Epidemiology followed up with patients.

Since the NM DOH Scientific Laboratory Division processes clinical, veterinary, and OMI samples from the entire state, the laboratory has a unique role in the detection of disease outbreaks and surveillance in New Mexico. The statewide picture of New Mexico's health can be observed from a centralized location and the excellent communication between the laboratory and the Office of Epidemiology facilitates timely outbreak detection and rapid response.

The 6th International Meeting on Microbiological Epidemiological Markers

Peter Gerner-Smidt, M.D., Dr.Sc., Statens Serum Institut, Copenhagen, Denmark; Cheryl Tar, Ph.D. and Bala Swaminathan, Ph.D., Foodborne and Diarrheal Diseases Laboratory Section, Centers for Disease Control and Prevention, Atlanta, GA

The sixth International Meeting on Microbiological Epidemiological Markers (IMMEM6) was held at the winter resort of Les Diablerets in Switzerland from August 23-26, 2003. The IMMEM is held every three years and all six meetings thus far have been held in Europe. The IMMEM focuses entirely on typing of and typing methods for infectious disease agents, with particular emphasis on the epidemiology of human and veterinary pathogens. Because of the narrow focus, the IMMEM is usually a small meeting with attendance in the 100 to 200 range, thus maximizing interactions and stimulating discussions among the delegates. This year, IMMEM6 had a record attendance of 350 delegates.

Presentations at previous IMMEMs provide a chronological history of the evolution of molecular typing methods. Typing methods in routine use today, such as plasmid profiling, microrestriction analysis, ribotyping, pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis, amplified fragment length polymorphism, and multilocus sequence typing were introduced to many of the participants for the first time in previous IMMEMs.

High throughput Single Nucleotide Polymorphism (SNP) analyses and Multi Locus Variable number tandem repeat Analysis (MLVA) were the two newer typing approaches highlighted at IMMEM6. The SNP and MLVA typing methods require prior knowledge of sequence variation at specific genomic loci. Each SNP analysis detects polymorphisms at a single locus on the genome, and the determination of SNPs at multiple loci may provide sufficient information for strain typing. SNP analysis may be considered a simplified version of MLST, which catalogs nucleotide differences in the sequences of seven or more housekeeping genes. However, data acquisition for MLST is more time extensive, as one needs to sequence the fragments of the housekeeping genes under study. In contrast, for SNP-analysis one needs to detect the sequence variation only at a single nucleotide position; this may be indirectly assessed by real-time PCR with

concurrent melting point determination of the PCR-fragment or by using DNA arrays. PM Giffard from Australia described a software program which could identify informative SNPs from public domain MLST-databases. The *N. meningitidis* and the *S. aureus* databases were used as examples to show how the informative SNPs could be used to identify the different sequence types in the databases or offer the best discrimination between unrelated isolates.

While mutations in intergenic regions do not affect the amino acid sequence of genes, a mutation could affect gene regulation and therefore an iSNP may be under selective pressure.

Often, MLST and MLST-based SNP-analysis do not provide adequate discriminatory power for outbreak investigations; they are most useful for phylogenetic analyses or to track long-term epidemiological patterns. However, SNPs in regions outside the housekeeping genes may be useful to study short term relationships between strains, i.e. to identify outbreak strains. J Musser, USA, gave an overview of SNP-analysis and its utility for epidemiologic studies. Synonymous SNPs (sSNPs) are SNPs that occur in genes but do not result in amino acid changes when translated. These are useful for inferring the phylogenetic relationships between closely related strains. Non-synonymous SNPs (nsSNPs) are SNPs that result in amino acid changes when



Les Diablerets Resort in Switzerland

translated, thus resulting in an altered protein sequence and possibly also in altered protein function; for this reason, nsSNPs are often under selective pressure and therefore are not reliable for phylogenetic analysis. A special variant is the iSNP, which is an SNP in an intergenic region. The utility of this type of SNP may be similar to nsSNP. While mutations in intergenic regions do not affect the amino acid sequence of genes, a mutation could affect gene regulation and therefore an iSNP may be under selective pressure.

The MLVA method has been discussed in previous issues of PulseNet News and is under development as second generation PulseNet methods for *E. coli* O157, *Salmonella*, and *Listeria monocytogenes*.

WP Terletski from Germany introduced a method he termed Subtractive Restriction Fingerprinting. This is basically a variation of microrestriction analysis. Purified DNA is digested using two enzymes; then two oligonucleotides that match the recognition sequence of each restriction enzyme are labelled with biotin or Dig-UTP, and are ligated to the restriction fragments. The fragments now labelled with biotin are removed by the use of streptavidin coated beads, leaving only the fragments labelled with Dig-UTP in both ends in solution. These are then separated in a gel and visualized using standard Dig-visualisation technology. In this way the resulting fragments show equal intensity because

(Continued on page 6)

STATE, COUNTY AND CITY HEALTH DEPARTMENTS

State, County and City Public Health Laboratories

From around the nation, we welcome:

- **Cathy Adams** who will be running PFGE at the San Diego Public Health Laboratory
- **Shawn Lee** who will be running PFGE at the New Jersey Department of Health
- **Eric Casey** who will be running PFGE at the Texas Department of Health.

Farewells

- **Arlene Lefebvre**, from the Department of Health & Hospitals Central Laboratory in New Orleans, LA, is no longer working with PFGE.
- **Linda J. Nims, M.S.**, the Supervisor for the General Microbiology Scientific Laboratory Division at the New Mexico Department of Health, is retiring after 26 years of dedicated service.



Workshop Participants and Instructors in the lobby of the Commonwealth of Virginia Laboratories

Food Industry *(Continued from page 3)*

maintained by Dr. Martin Wiedmann's Laboratory of Food Microbiology and Pathogenesis of Foodborne Disease.

In his introductory remarks, Dr. Bala Swaminathan, Chief, Foodborne and Diarrheal Diseases Laboratory Section, CDC, identified the establishment of key partnerships as the next frontier for PulseNet. A partnership between PulseNet and the food industry could potentially allow industry access to current information on prevalent DNA fingerprints of pathogenic strains involved in human disease, facilitate the establishment of monitoring programs by the food industry for outbreak associated strains, and improve the industry's ability to assess the likelihood of



Students hard at work!

involvement of specific food processing plants in outbreaks under investigation based on comparisons of outbreak pattern(s) with plant monitoring data. If obstacles to this kind of collaboration can be overcome, the public health sec-

tor could benefit from information from the food industry on the association of specific subtypes of foodborne pathogens with specific food types, particularly in early stages of outbreak investigations. This collaboration and exchange of information would improve the ability of public health agencies to assess the effectiveness of control and prevention programs for foodborne diseases. **CDC**

ACKNOWLEDGEMENTS.

This workshop was made possible by the special efforts of Dr. Bala Swaminathan and Dr. Les Smoot, Director of Food Safety with Nestlé USA, Inc., and Chair of the ILSI N.A. Technical Committee on Food Microbiology. Funding for this workshop was provided by the ILSI N.A. Technical Committee on Food Microbiology, with special contributions by Campbell Soup Company, CTI Foods, Gerber Products Company, General Mills, Inc., H.J. Heinz Company, Kraft Foods NA, Nestlé USA, Inc., The Procter & Gamble Company. Other contributors included Cornell University and the National Food Processors Association.

PULSENet DATABASE DEVELOPMENT: PROGRESS AND PLANS

Susan B. Hunter, M.S. and Jennifer Kincaid (Foodborne and Diarrheal Diseases Branch) Centers for Disease Control and Prevention, Atlanta, GA

The PulseNet Database Development team provides the following update on efforts to improve the PulseNet databases and client scripts. Overall, recent efforts have been focused on 3 goals:

1. Development of client scripts and on-line database for *Shigella*.
2. Transitioning the *E. coli* database to SQL format.
3. Changing to a master script format for PulseNet scripts.

Progress on each of these goals impacts the progress made on the others.

During the development of the *Shigella* scripts and the conversion of the national *Shigella* database to the on-line format, we decided to create the database in

SQL format. The database team is testing the SQL-formatted database at this time. The scripts should be ready for first round testing very soon.

The first step in formatting the *E. coli* database to SQL was converting the current database to a SQL test system. The second step was testing a new script for uploading data to a SQL format database. The PulseNet Database Development Team thanks the volunteers who have tested various versions of this script and instructions for installation and use. The new scripts allow the user to upload multiple lanes from a single TIFF to a SQL-formatted national database. This improvement will allow quicker uploading to the national database.

Another improvement is the transition to a master script format for scripts development and distribution. All client scripts will become part of a master script set



with modifications as needed for specific organisms. This will allow the development

team to more uniformly upgrade all client script sets. The master script format will also be more cost effective for future improvements. Many of the improvements that were/will be added are due to requests from the PulseNet participants. Please continue to let us know what changes and additions you would like to see. Also, let us know when you encounter errors or problems by sending an email to PFGE@CDC.GOV or calling (404) 639-4558. By improving the client scripts, we can make the national data easier to access and work with.

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National Center for Infectious Diseases,
Division of Bacterial and Mycotic Diseases,
Foodborne and Diarrheal Diseases Branch.

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Epidemiological Markers (Continued from page 4)

the band intensity is only dependent on the copy number of fragments and not on the size of the fragment as in an ethidium bromide stained gel. With careful selection of restriction enzymes up to 50 fragments may be visualized on a gel after ordinary electrophoresis. The enzyme combination *EcoRI*-*PvuII* was found suitable for the typing of zoonotic salmonella serotypes. The entire procedure up to electrophoresis is completed within 4 hours and the method compares favourably with PFGE in this respect. However, because restriction-based typing methods are being replaced by amplification and sequencing-based methods, this method is unlikely to get the recognition it might have achieved, had it been invented 10 years ago.

Despite the high level of interest in new subtyping methods, a workshop on PFGE drew a capacity crowd at the meeting. Dr. Richard Goering and Dr. Marc Struelens led the discussions and responded to questions and comments from the audience. PulseNet protocols were frequently mentioned in the discussions

and were generally accepted as benchmarks for PFGE subtyping.

For the first time, the American Society for Microbiology assisted in the organization of IMM7. IMM7 will move across the Atlantic to Victoria, British Columbia, Canada, in 2005. The organizers of IMM7 are extremely interested in holding the IMM7 meeting in conjunction with the PulseNet annual meeting with one day of overlap between the two meetings. The proposal merits serious consideration

by PulseNet meeting organizers because this will give an opportunity for cross-fertilization of ideas and subtyping strategies between the two groups. **CDC**

INSIDE PulseNet
Visit <http://www.cdc.gov/pulsenet> for the latest...

Publications and Abstracts

- McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed Field Gel Electrophoresis typing of Oxacillin-Resistant *Staphylococcus aureus* isolates from the United States: Establishing a national database. *J Clin Microbiol* 2003 Nov;41(11):5113-5120.

CDC PulseNet Task Force New Members PulseNet National Database Administration Team:

- **Nehal Patel** joined the PulseNet database administration team in

November 2003. Nehal graduated from the University of Georgia in August 2003, with a B.S. in Biology. She will be working on a project funded by USDA, involving the analysis and data management of *Salmonella* PFGE patterns.

- **Beth McGlinchey**, a USDA employee, will be working in conjunction with the PulseNet database administration team to create and establish a *Salmonella* database of PFGE patterns from USDA isolates. Beth attended Georgia College and State University, receiving her B.A. in 2001 and her M.S. in Biology in May 2003.